Examining the effect of Cumulative Low Dose Radiation on TK6 Human Lymphoblastoid Cells During Simulated Microgravity

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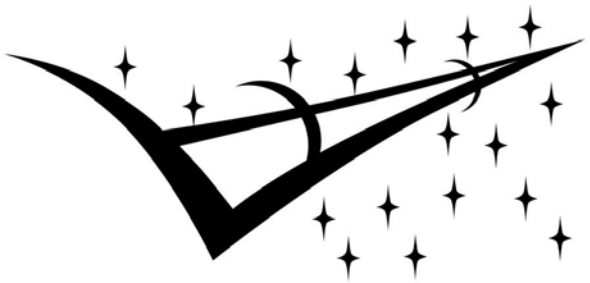


Introduction

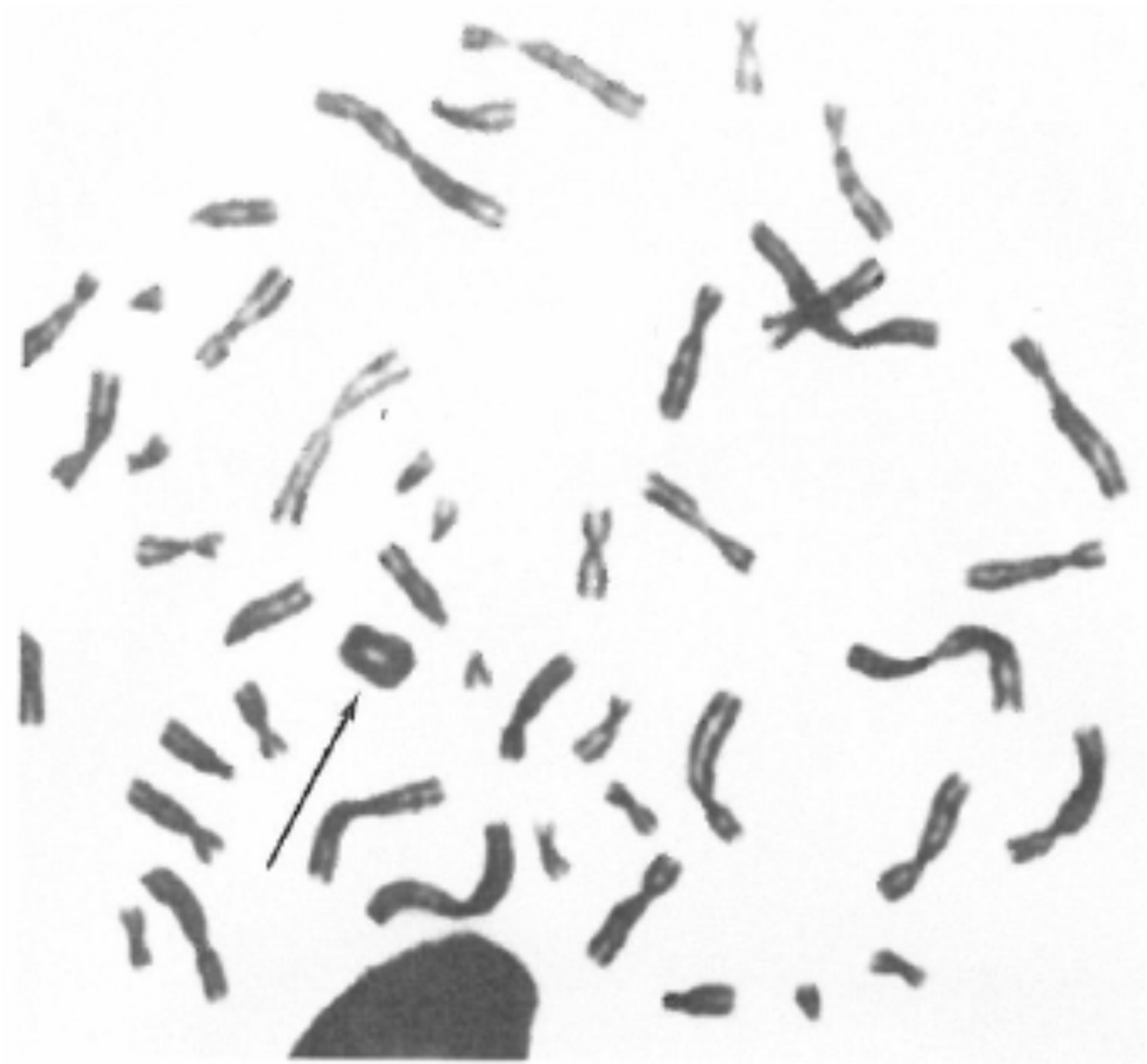
Background

- Many researchers study the effect of microgravity and space irradiation separately
- Most space radiation research is currently focused on a single high dose of radiation.
- The effect of cumulative low-dose irradiation on cell damage is not well known.

Objectives of Internship

- Our goal was to assess the combined effect of continuous low-dose radiation during simulated microgravity conditions.

Mechanism of Radiation Damage



Introductory Terminology: Absorbed Dose

- Gray = the SI unit of absorbed dose
  = 1 Joule of radiation absorbed / Kilogram

- Rad = (Radiation Absorbed Dose) the old unit of absorbed dose
  = 0.01 Joule of radiation absorbed / Kilogram

One Gray = 100 Rads

Introductory Terminology: Equivalent Dose

- Sievert : Is a measure of equivalent / effective dose.

  Each tissue is assigned a weighting factor

  Sievert = Gray x weighting factor

- Rem = (roentgen equivalent in man) the old unit of equivalent or effective dose

  = Rad x weighting factor

One Sievert = 100 REM

Radiation Weighting Factor

Radiation Weighting Factors

Type and Energy Range	Radiation Weighting Factor, W_R
Photons	1
Electrons	1
Protons	2
α-Particles, fission fragments, heavy nuclei	20
Neutrons	A continuous curve is recommended with a maximum of 20 for the most effective neutrons of about 1 MeV

Tissue Weighting Factor

Tissue Weighting Factors

Tissue	W_T	ΣW_T
Bone marrow, breast, colon, lung, stomach	0.12	0.60
Bladder, esophagus, gonads, liver, thyroid	0.05	0.25
Bone surface, brain, kidneys, salivary glands, skin	0.01	0.05
Remainder tissues[a]	0.10	0.10

Equivalent Dose

- Equivalent Dose = Σ Absorbed dose x W_R x W_T

Materials and Methods

- TK6 Human Lymphoblastoid cells
  - Sensitive to radiation
- Hardware
  - Low Dose Gamma Ray Source (Cesium - 137)
  - High Aspect Ratio Vessel (HARV)
  - Rotary Cell Culture System (RCCS)
- Experiment Design

Experiments 1-5, 9:	40,000 cells/mL for 3 days
Experiments 6a:	80,000 cells/mL for 2 days
Experiments 6b, 7, 8	80,000 cells/mL for 3 days

Setup

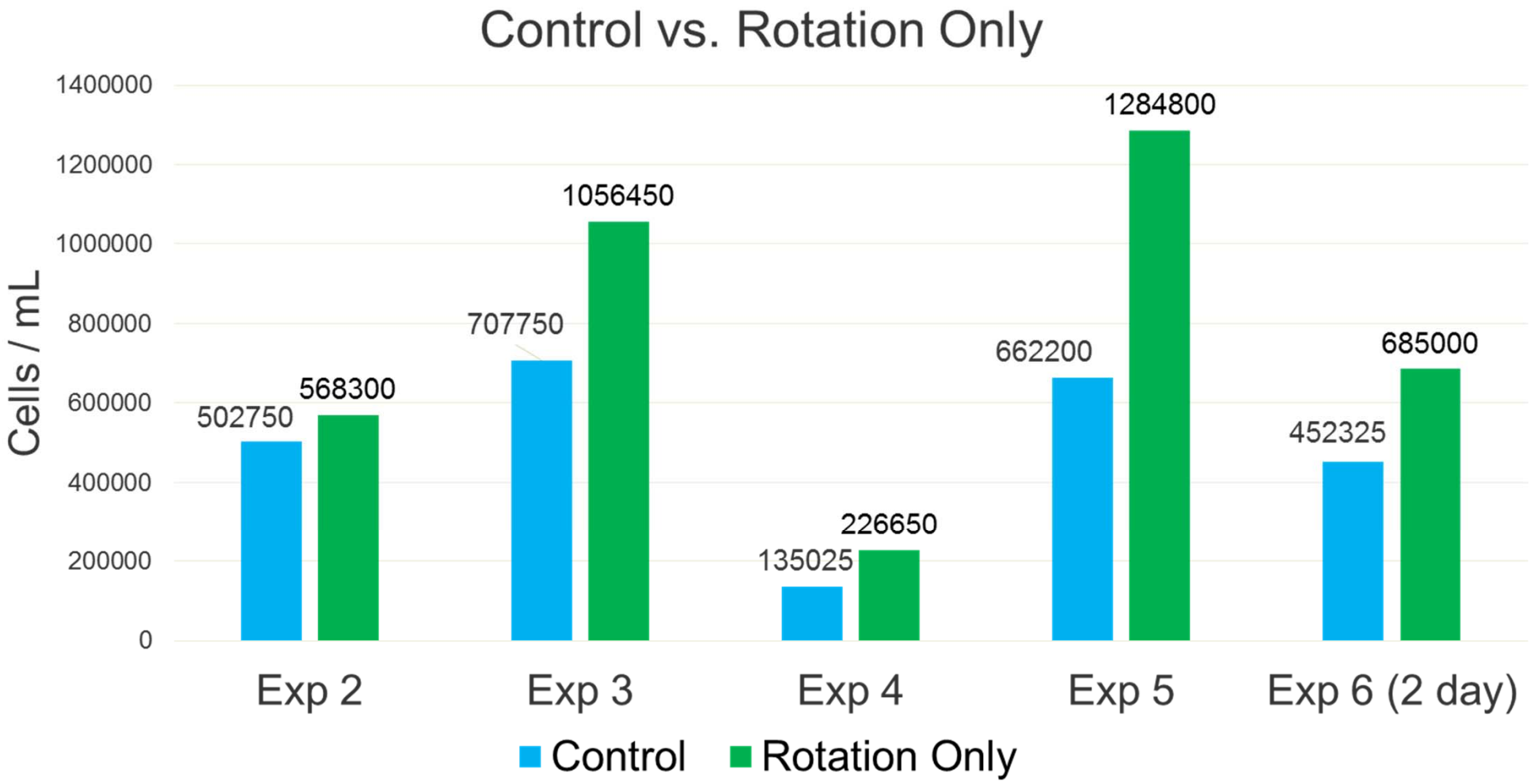

CAUTION
HIGH
RADIATION AREA
FILM BADGE
REQUIRED
KEEP OUT
Irradiation in
Progress

Results

- 9 Experiments were conducted
- Data was gathered from 5

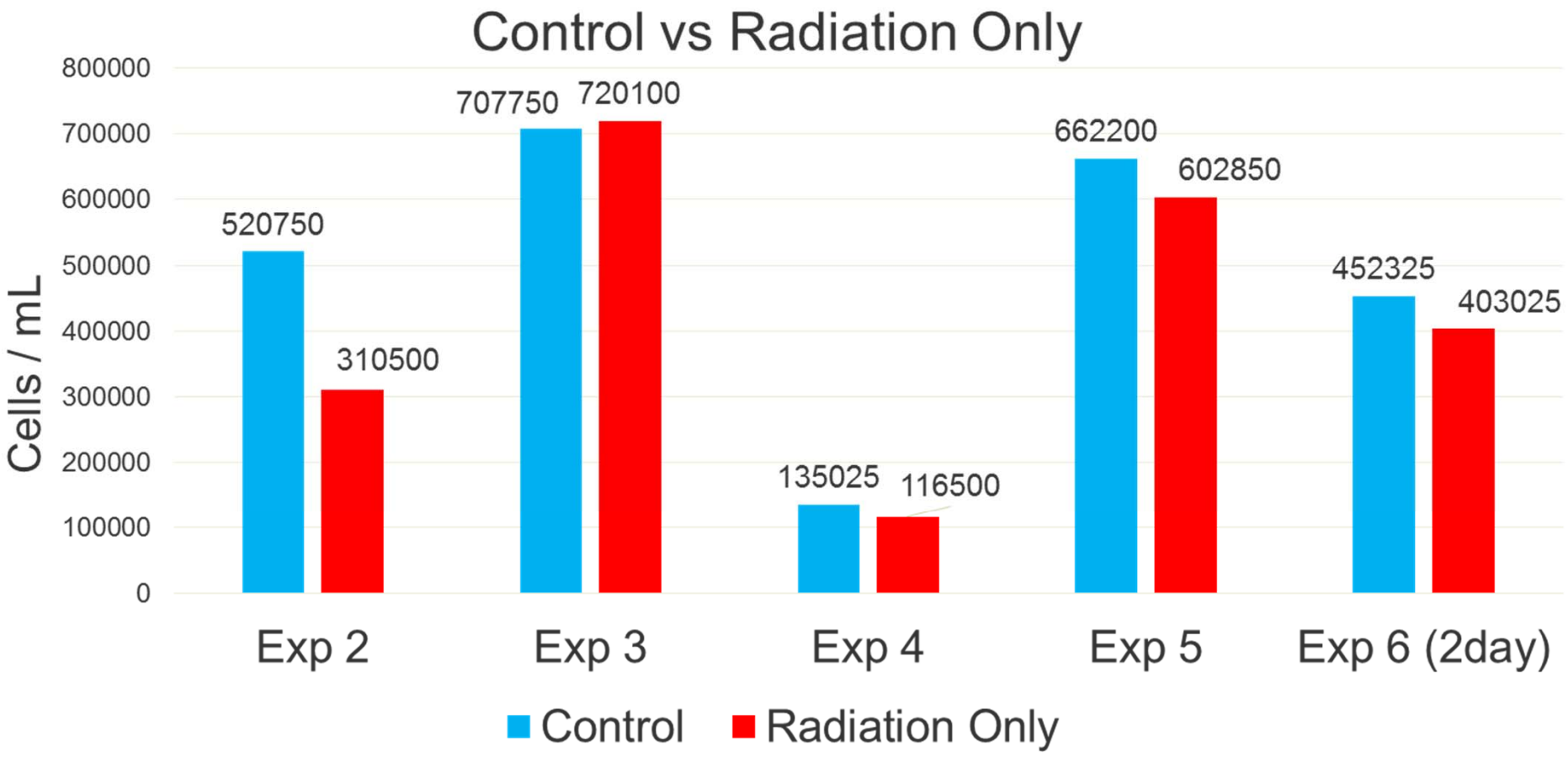
Experiment	Outcome
1	Power surge caused radiation to stop
2	Usable
3	Usable
4	Usable
5	Useable
6a	Useable
6b	Rotation device stopped working
7	Failure to proliferate
8	Failure to proliferate
9	Harvested for future RNA analysis

Results



Average increase in Rotation Only group vs Control = 1.55x ± 0.13

Results



Average percent decrease due to Radiation vs Control = 14.01 ± 6.59

Results

- Predict the expected cell numbers for the combined effect of rotation & radiation based upon previous data
- Example: Experiment 2

  Control = 502,750 cells/mL

  x 1.13 (rotation growth factor observed compared to control)

  = 568,300 cells/mL

Reduce 568,300 by 38.24% (radiation damage observed compared to control)

= **350,982 cells/mL**

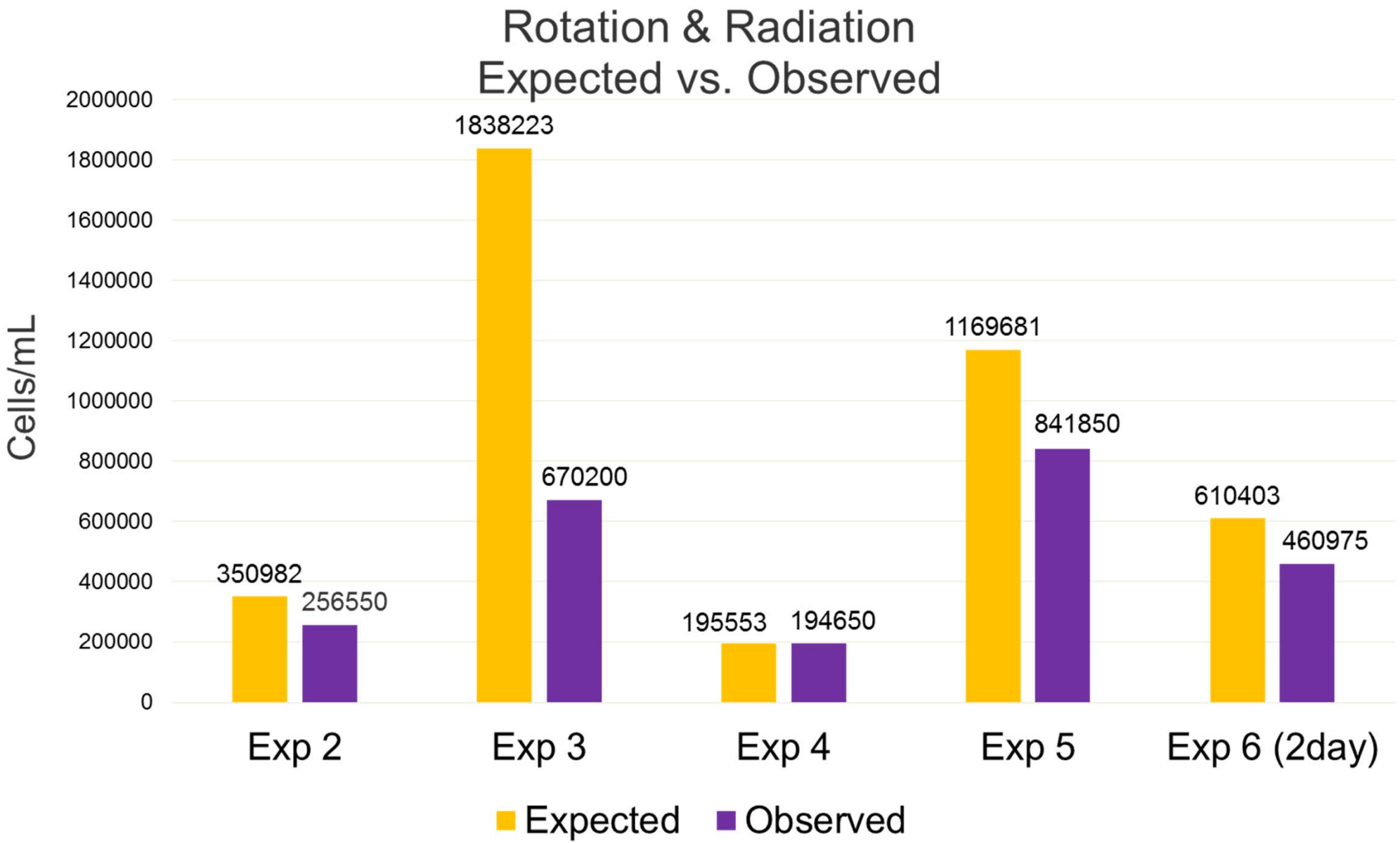
(Expected effect of combining rotation & radiation together)

Results

Experiment	Expected (cells/mL)	Observed (cells / mL)
2	350,982	256,550
3	1,838,223	670,200
4	195,553	194,650
5	1,169,681	841,850
6a	610,403	460,975

An Average of 28.68 □ 10.08 % Lower than Expecte

Results


Rotation & Radiation
Expected vs. Observed
Cells/mL
2000000
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
350982
256550
1838223
670200
195553
194650
1169681
841850
610403
460975
Exp 2
Exp 3
Exp 4
Exp 5
Exp 6 (2day)
Expected
Observed

Discussion

- The data suggests a **synergistic effect** exists when Rotation (simulated microgravity) and Radiation are combined together

- Current models for determining upper limit radiation doses are based upon terrestrial models.

- Weighing factors:

$$\text{Equivalent Dose} = \Sigma \text{ Absorbed dose} \times W_R \times W_T$$

Discussion

- Perhaps there is a heretofore undiscovered "third weighting factor," a so-called "gravity weighting factor: W_G."

- Equivalent Dose =

  Σ Absorbed dose x W_R x W_T x $\mathbf{W_G}$

Discussion: Caveats

1. The cell numbers differed substantially from experiment to experiment
2. A rotating bioreactor can only incompletely approximate microgravity
3. Cells may have been dying due to other reasons
   - E.G. Stress from presumably being packed together?
   - Currently testing for oxidative stress markers (GSH, LPO)
4. Radiation damage was not completely assessed via micronuclei analysis (currently underway)

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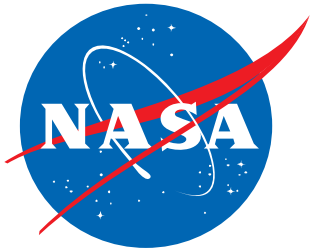
NASA